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NEPHROLOGY FORUM

Mechanisms of glomerular injury in immune-complex disease

Principal discussant: WILLIAM G. COUSER

University of Washington, Seattle, Washington, USA

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Case presentation

A 27-year-old black woman was admitted to Harborview Medical Center, a component of the Warren G. Magnuson Health Services Center at the University of Washington, complaining of midepigastric pain, chills, and sweats for the previous two days. She had been hospitalized several times previously for similar complaints. Each time the pain resolved spontaneously and no cause was found. The patient also had a history of intravenous drug abuse including heroin and amphetamines over a period of 7 years, but she denied drug use in the 2 months preceding admission. Her most recent medical evaluation was carried out 4 months prior to the present admission. At that time her serum creatinine was 0.9 mg/dl, BUN was 24 mg/dl, urinalysis was negative for protein, and urine sediment was unremarkable.

Physical examination revealed a blood pressure of 160/110 mm Hg, temperature of 38°C, and pulse of 90/min. Multiple healed needle tracks were present on both arms. The ocular fundi were normal. Dental hygiene was poor. Mild, nontender cervical adenopathy was present. The lungs were clear. A grade III/VI crescendo-decrescendo systolic murmur was noted at the left sternal border radiating to the apex. The liver was palpable 1 cm below the right costal margin, but the spleen was not felt. Bilateral 1+ pretibial edema was present. No Roth spots or splinter hemorrhages were noted.

Laboratory studies demonstrated an iron deficiency anemia, with a hematocrit of 30% and a reticulocyte count of 2.0%. The corrected erythrocyte sedimentation rate was 48 mm/hr. The white blood cell count was 21,600/mm^3 with 81 neutrophils, 3 bands, 10 lymphocytes, 5 monocytes, and 1 eosinophil. The platelet count was 460,000/mm^3. Hemoglobin electrophoresis was normal. Urinalysis disclosed 4+ protein with no glucose; the urine sediment contained 10 to 20 red blood cells and 5 to 10 white blood cells per high-power field, numerous granular casts, and occasional red cell and fatty casts. Urine protein excretion was 10.9 g/24 hr. Total serum protein was 7.9 g/dl with albumin of 3.0 g/dl. There was a diffuse increase in gamma globulins. Serum creatinine was 1.0 mg/dl; BUN, 15 mg/dl; and fasting blood sugar, 92 mg/dl. Serum sodium, potassium, chloride, bicarbonate, calcium, phosphate, uric acid, cholesterol, and liver function tests were within normal limits. Antibody was present to hepatitis B core and surface antigens, but the test for hepatitis B surface antigen was negative. Circulating immune complexes measured by a solid phase C1q binding assay were 27% (normal, 0%–9%). Serum C3 was 134 mg/dl (normal, 100–200), C4 was 25 mg/dl (normal, 10–40), and CH_{50} was 116 units (normal, 80–160). An antinuclear antibody test was positive in a speckled pattern at 1:10. Tests for rheumatoid factor, streptozyme, and cryoglobulins were normal or negative. Toxic screens were negative for narcotics and amphetamines. Ten blood cultures were sterile. Chest x-ray and electrocardiogram were unremarkable. Echocardiography revealed no valvular vegetations or thickening. Ultrasound examination revealed the kidneys to be of normal size.

Following hospitalization, the patient's blood pressure returned to normal, and the heart murmur disappeared following short-term treatment with diuretics and propranolol. Proteinuria and hematuria persisted, however, and the white blood cell count rose to 31,600/mm^3 and serum creatinine increased to 2.5 mg/dl. A percutaneous renal biopsy revealed a diffuse proliferative glomerulonephritis with cellular crescents in 50% of glomeruli. Glomeruli without crescents showed mesangial thickening and increased numbers of neutrophils and macrophages in capillary loops. Two small foci of necrosis were noted. The interstitium contained a significant infiltrate of mononuclear cells without scarring or fibrosis. Many tubules contained red blood cell or proteinaceous casts. Immunofluorescence revealed diffuse, coarsely granular deposits of IgG, IgM, C3, and C1q in the mesangium and along the capillary walls, but IgA was absent. Electron microscopy showed extensive subendothelial and mesangial electron-dense deposits most prominent near the mesangial waists as well as scattered electron-dense deposits on the subepithelial surface of the capillary wall with extensive effacement of the epithelial cell foot processes.

Following reports of the negative blood cultures, negative echocardiogram, and renal biopsy, an extensive search was undertaken for other foci of infection. Dental examination revealed multiple decayed teeth as well as two large and several small gingival abscesses. Eight teeth were extracted, the abscesses were drained, and the patient received a 3-week course of intravenous and oral antibiotics. At discharge, the serum creatinine was 1.2 mg/dl, urine sediment was free of red blood cells and red blood cell casts, and urine protein exretion was 4 g/day. On a followup visit to the renal clinic 4 months later, the serum creatinine was 1.1 mg/dl, and urine protein excretion was 1.9 g/day.

Discussion

DR. WILLIAM G. COUSER (*Head, Division of Nephrology, and Professor of Medicine, University of Washington, Seattle, Washington*): Several renal lesions have been reported in patients, such as this one, who have a history of parenteral drug abuse. These lesions include nephrotic syndrome with focal glomerular sclerosis [1], hepatitis B-associated membranous nephropathy [2], vasculitis [3], interstitial nephritis [4], and amyloidosis [5]. But this patient presents a typical picture of a chronic postinfectious glomerulonephritis associated with gran-

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ular immune-complex deposits at mesangial, subendothelial, and subepithelial sites. Similar deposits are seen in other postinfectious nephropathies, such as subacute bacterial endocarditis and shunt nephritis as well as in diseases such as lupus nephritis, type-I membranoproliferative glomerulonephritis (MPGN), and severe IgA nephropathy or Henoch-Schönlein purpura [6]. The pyogenic gingival infection that initiated this patient's illness probably arose from mixed flora containing aerobic and anaerobic streptococci and other organisms. Although postinfectious nephritis usually is associated with group-A beta-hemolytic streptococci of so-called nephritogenic serotypes, it also has been reported with non-group-A streptococci as well as with a variety of other organisms [7–9].

The association between bacterial infection and glomerulonephritis was first identified by Lohlein in 1907 [10]. Schick first suggested an immune basis for glomerulonephritis after observing that nephritis following scarlet fever or tonsillitis occurs after a time interval consistent with that seen in hypersensitivity reactions [11]. Von Pirquet came to similar conclusions after observing the similarity between the time course of poststreptococcal nephritis and that of serum sickness in humans [12]. Support for this hypothesis was provided in the late 1950s by the first immunofluorescence studies of human kidney tissue, which demonstrated granular immune deposits in diseased glomeruli in poststreptococcal glomerulonephritis and other diseases [13]. Experimental verification of the immune basis of nephritis following exposure to a foreign protein antigen was provided by the pioneering studies of Germuth and Dixon in the 1950s and 1960s in acute and chronic BSA-serum sickness in rabbits [14–17]. These investigators noted that clinical and histologic manifestations of glomerulonephritis could be induced in most rabbits 1 to 2 weeks following a single injection of BSA, and that immune deposits of antigen and antibody developed at mesangial, subendothelial, and subepithelial sites just as in the patient we are discussing [14–16] (Fig. 1). It was further noted that the glomerular deposits developed coincident with the appearance of circulating, soluble immune complexes of the same antigen and antibody, and that the site of deposit formation was related to the immune response and consequent ratio of antigen to antibody present in the circulation [17, 18]. Thus animals with high antibody levels formed large, lattice-like, insoluble immune complexes that were cleared rapidly by the mononuclear phagocyte system (MPS) or were deposited in small amounts in the glomerular mesangium [15, 16]. A less vigorous antibody response led to smaller, more soluble complexes, with more deposits in the mesangium and along the capillary wall at subendothelial sites [15–18]. Poor antibody responders, or animals repeatedly re-injected with antigen to maintain persistent antigen excess, developed predominantly subepithelial deposits [16–18]. Because the deposits formed only in the presence of circulating immune complexes, and because antigen and antibody could not be detected alone in glomeruli by conventional immunofluorescence techniques, it was concluded that the granular deposits and glomerulonephritis resulted from the glomerular trapping of soluble immune complexes formed in the circulation. The site of complex localization was believed to be determined largely by the ratio of antigen to antibody and hence the size of the lattice of the immune complexes formed [16–18]. The glomerulus was seen as only a passive filter in this process. This view of the

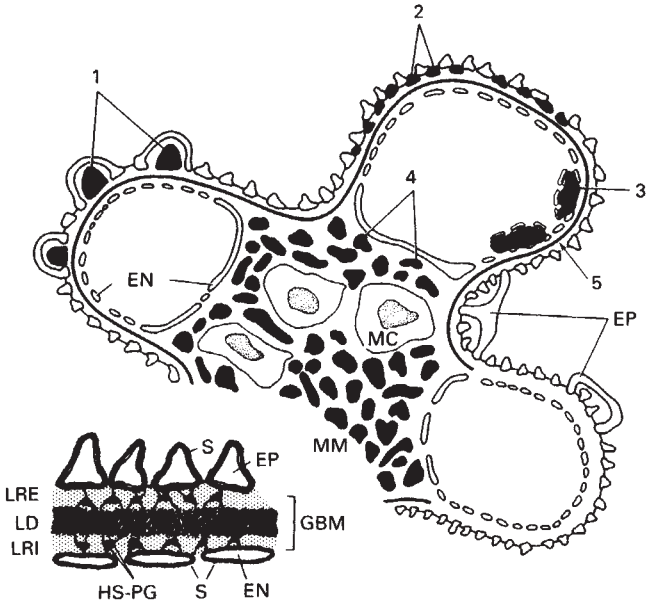

Fig. 1. *Schematic depiction of intraglomerular sites of immune-complex formation.* Subepithelial deposits such as those in postinfectious glomerulonephritis (1) or membranous nephropathy (2) apparently form by local, or in-situ, mechanisms. Subendothelial (3) and mesangial (4) deposits are usually seen together and may form locally or result from the passive trapping of preformed immune complexes from the circulation. Anti-GBM antibody deposits are in a linear pattern within the capillary wall itself (5). The mechanism of formation, composition, quantity, and relative accessibility of each of these deposits to circulating inflammatory cells are the major determinants of the type of lesion produced.

The inset illustrates the three layers of the normal glomerular capillary wall, endothelial cells (EN), GBM, and epithelial cells (EP). The negative charge on the capillary wall results from the sialoproteins (S) coating the endothelial and epithelial cell surfaces and the heparan-sulfate proteoglycan (HS-PG) anionic sites in the lamina rara interna (LRI) and externa (LRE) of the GBM. (From Ref. 44).

pathogenesis of immune-complex nephritis, prevalent for nearly two decades, was supported by a number of studies of the fate of preformed immune complexes infused into animals (reviewed in 19). These studies demonstrated that mesangial as well as subendothelial deposits could be produced by circulating complex trapping. The origin of subepithelial deposits was not clarified by these studies, however. Moreover, short-term infusion of preformed immune complexes did not induce glomerular disease sufficient to permit studies of how this process caused glomerular injury, although inflammatory cell infiltrates were sometimes seen [19]. Concepts of the mediation of immune-complex–induced tissue damage therefore were derived primarily from studies in experimental models of nephrotoxic, or anti-GBM, nephritis and were then extrapolated to glomerular injury due to preformed immune-complex trapping [20].

Interest in the pathogenesis of immune-complex glomerulonephritis was rekindled in the late 1970s. In Heymann nephritis, a rat model of membranous nephropathy induced by active or passive immunization against a proximal tubular brush-border antigen (Fx1A), pretreatment with the aminonucleoside of puromycin, an epithelial cell toxin that interferes with glycopro-

tein turnover on the cell membrane, prevented the formation of subepithelial immune-complex deposits [21–23]. This finding first implicated a property of the glomerulus itself in immune-complex formation. It was then found that the deposits in the Heymann models, previously believed to result from trapping of circulating immune complexes containing brush-border antigens [24], could be produced by antibody binding directly to an intrinsic glomerular antigen [25, 26]. Subsequent studies have extensively reevaluated the nature of glomerular immune-complex deposits at mesangial, subendothelial, and subepithelial sites [27, 28] (Fig. 1), and have considerably revised and extended our understanding of how these deposits form. An outline of the mechanisms of glomerular immune deposit formation in the order I will discuss them here is presented in Tables 1 and 2. Figure 2 illustrates the mechanisms that mediate immune glomerular injury.

Table 1. Mechanisms of subepithelial immune-complex formation

I. In-situ immune-complex formation
- A. Antibody binding to glomerular antigens
  1. Heymann Antigen [25, 26, 30]
  2. Epithelial cell foot process antigen [38]
- B. Planted nonglomerular antigens
  1. Charge-dependent mechanisms
     a. Cationic antigens [47–49, 62]
     b. Anionic antigens [66, 80]
  2. Charge-independent mechanisms
     a. Direct binding of antigens to capillary wall by underfined mechanisms [36, 83]
     b. IgG binding to capillary wall by immune mechanisms [84, 85]

II. Circulating immune-complex trapping
- A. ? Cationic or low avidity immune complexes [65, 87, 89]

Mechanisms of subepithelial glomerular immune-complex formation

In-situ immune-complex formation

Fixed glomerular antigens. Subepithelial immune-complex deposits are now believed to form primarily on a local, or in-situ, basis rather than from circulating immune-complex trapping, but this mechanism may involve either insoluble fixed renal antigens or soluble exogenous antigens. Table 1 lists the mechanisms involved. The in-situ mechanism has been best studied in the rat, where it was first shown to be responsible for initiating subepithelial deposits in the passive Heymann nephritis model [25, 26]. The antigen responsible for this lesion, which bears a striking resemblance to human idiopathic membranous nephropathy, is now believed to be a glycoprotein with a molecular weight of about 330 Kd (GP330) [29, 30]. The antigen is distributed along the glomerular epithelial cell membrane, where it is localized in endoplasmic reticulum and in coated pits [30]. Unlike deposition of anti-GBM antibody, which occurs almost immediately [31], antibody to the epithelial cell antigen deposits slowly for reasons that are unclear [32]. Studies of antibodies combining with similar plasma-membrane antigens (such as angiotensin converting enzyme) on pulmonary endothelial cells or in the oolemma of rabbit oocytes [33, 34], and studies utilizing antibody to GP330 and cultured rat glomerular epithelial cells [35] have suggested that the interaction of divalent antibody with such plasma membrane antigens induces antigen redistribution and capping on the cell surface. Immune complexes are then shed into the adjacent lamina rara externa of the GBM and into slit pores. This distribution probably results in the discontinuous, finely granular appearance of these deposits. Other mechanisms may contribute to immune-complex formation in Heymann nephritis. For example, Abrass and Cohen have identified a component of the nephritogenic tubular brush-border antigen that can localize directly in glomeruli from the circulation and which may serve as a "planted" antigen [36]. These workers also have described the development later in the course of passive Heymann nephritis of a second antibody that reacts with glomeruli but not with brush borders [37]. Another example of a spontaneous nephropathy due to antibody reacting with an apparently different epithelial cell antigen has been reported in rabbits [38].

Table 2. Mechanisms of mesangial and subendothelial immune-complex formation

In-situ immune-complex formation
- Glomerular antigens
  - Endothelial cell-membrane antigens [90][a]
- Planted nonglomerular antigens
  - Charge-related localization of large cationic antigens [63]
  - Biochemical affinity of antigen for GBM glycoprotein [93]
  - Mesangial uptake of macromolecular antigens [97]
  - Immune-complex deposit interaction with:
    1. Rheumatoid factors [103]
    2. Antiidiotypic antibodies [104]
    3. Circulating immune complexes [105]

Circulating immune-complex trapping
- Determinants of:
  - Systemic factors
    1. Renal blood flow [110]
    2. Mononuclear phagocyte system function [107, 108]
    3. Erythrocyte CRl receptors [109]
  - Glomerular factors
    1. Hemodynamic changes [111]
    2. Charge and permeability [78, 79]
    3. Mesangial afferent and efferent limb function [61, 112]
  - Properties of immune complexes
    1. Size [113]
    2. Charge [89, 114]
    3. Complement-fixing ability [109]
    4. Biodegradability [99, 101].

[a] Numerals in brackets are reference numbers.

Since the immunogen usually utilized to elicit Heymann nephritis is derived from a crude proximal tubular brush-border membrane fraction, many laboratories have searched for evidence of brush-border antigen or antibody to the immunogen in human membranous nephropathy. With rare exceptions [39, 40], these studies have been negative [41, 42]. However, studies designed to evaluate the possibility that the antibody in human membranous nephropathy may be directed against a glomerular epithelial cell antigen rather than against a tubular brush-border antigen have not yet been carried out despite increasing suspicion that such an autoimmune mechanism is probably operative in this disease [43, 44].

Except for anti-GBM disease, no human equivalent of immune-complex glomerulonephritis due to antibody reacting with a fixed glomerular antigen has so far been identified.

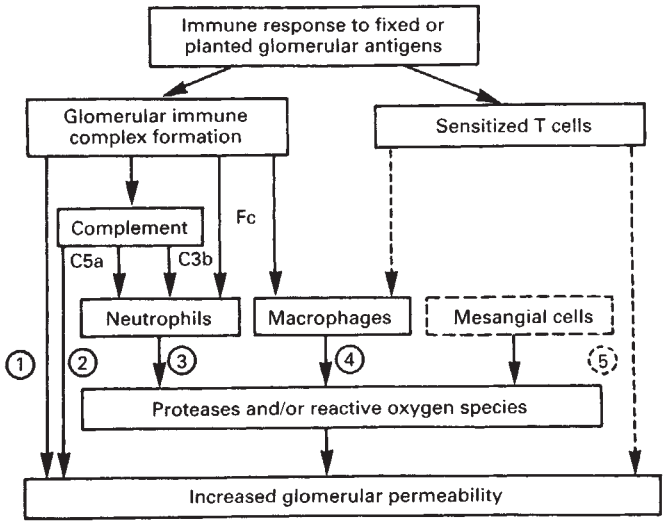

Fig. 2. *Defined mechanisms by which glomerular immune-complex deposits mediate tissue injury as evidenced by increased glomerular permeability and proteinuria are shown in solid lines.* Proteinuria may result from antibody binding to some fixed glomerular antigens (1), or from the direct effect of complement activation (2). Complement activation by subendothelial and mesangial deposits may attract neutrophils by chemotactic (C5a) or immune adherence (C3b) mechanisms (3). Macrophages may also be attracted by immune adherence mechanisms involving Fc receptors (4). The capacity of sensitized T cells to cause glomerular injury by macrophage recruitment, or perhaps directly (5), is not yet fully established and is therefore shown in dotted lines. Similarly, the role of mesangial cells in producing glomerular injury remains hypothetical. Activated inflammatory cells cause basement membrane damage by release of proteases and/or reactive oxygen species.

However, antibodies to DNA are known to be polyspecific and reactive with shared antigenic epitopes on several structures including polynucleotides, phospholipids, cell membranes, cytoskeletons, and bacteria [45]. Monoclonal anti-DNA antibodies have been shown by Madaio et al to bind directly to glomerular structures in mice [46]. Such studies support the hypothesis that antibody binding to fixed non-GBM glomerular antigens may contribute to some types of human immune-complex nephritis.

Planted non-glomerular antigens. Subepithelial immune-complex deposits also can occur in nephritis induced by exogenous antigens as they do in the chronic BSA serum-sickness models. Multiple mechanisms have now been identified that can lead to such "planted" antigen deposits (Table 1); several involve electrical interactions between glomerular anionic sites, principally the negatively charged heparan-sulfate proteoglycans in the laminae rarae of the capillary wall (Fig. 1), and cationic, or positively charged, immune reactants.

If chronic serum sickness is induced with BSA chemically modified to increase the pI from 4.5 to greater than 9.0, rabbits develop predominantly subepithelial deposits, independent of the immune response or of the quantity and size of circulating immune complexes [47]. Initial localization of cationic antigen prior to antibody binding has been demonstrated by both immunofluorescence and radiolabeling techniques, although the antigen does not persist and disease does not develop unless antibody binding produces immune complexes [47–49]. Moreover, a similar lesion can be produced if one perfuses an isolated kidney with cationic antigen followed by antibody to it, thus establishing that the deposits are formed locally [48, 49]. The granular nature of these deposits probably results from the tendency of anionic structures to coalesce after interaction with cationic molecules [50], as well as from the capacity of immune complexes formed of multivalent antigens and precipitating antibodies to rearrange and condense into larger latticed structures visible by electron microscopy [51].

Like Heymann nephritis, the cationic BSA serum sickness model has been suggested as a model of human membranous nephropathy [47, 52]. Thus therapy with polycations, which neutralize anionic sites and therefore reduce deposit formation in cationic BSA serum sickness, has been advocated for study in membranous nephropathy in humans [52]. As in all exogenous antigen-induced nephropathies, however, some deposits in serum sickness occur in subendothelial and mesangial sites as well [47, 53]. Deposits in these locations are atypical of idiopathic membranous nephropathy, which morphologically more closely resembles the lesion induced by the fixed-antigen mechanism. This distinction could have therapeutic relevance, because studies of polycation therapy in fixed antigen models of membranous nephropathy have shown no detectable effect on antibody deposition or on proteinuria [54].

This mechanism of cationic antigen-induced immune-complex nephritis recently has been applied to studies of postinfectious nephritis in humans. Vogt and colleagues have isolated several anionic and cationic extracellular proteins from nephritogenic streptococci and used specific antibodies to them to demonstrate glomerular localization of only cationic antigens in glomerular immune deposits in 8 of 18 patients with early poststreptococcal glomerulonephritis [55]. Evidence also has been provided for the participation of a streptococcal cell membrane antigen, endostreptosin, in immune deposits in patients with early poststreptococcal glomerulonephritis [56]. The deposits also may contain antibody to an abnormal IgG, desialated by the action of streptococcal neuraminidase, thereby making it more cationic [57, 58]. However, various inert macromolecules, such as streptococcal M proteins, may be localized in glomeruli without necessarily causing disease [59, 60]. Nonspecific uptake of circulating macromolecules is increased in damaged glomeruli [61]. Immunofluorescence studies that identify putative pathogenetic agents in human glomerular immune deposits therefore must be interpreted with considerable caution [62].

A second mechanism of in-situ subepithelial immune-complex formation related to charge involves large cationic antigens such as ferritin, which may first localize along the subendothelial surface of capillary walls. Secondary fixation of antibody leads to formation of large latticed subendothelial deposits [63, 64]. These complexes subsequently can dissolve and individual reactants (or perhaps small immune complexes) can cross the capillary wall to reform as larger immune complex aggregates in the subepithelial space [63]. This mechanism may be operative in diseases in which subepithelial and subendothelial deposits coexist, as in the patient discussed here, or in patients with severe lupus nephritis.

Although most attention has been directed at glomerular localization of cationic antigens, anionic antigens such as native

BSA and DNA clearly are important in producing both experimental and clinical immune-complex nephritis. Two additional charge-related mechanisms may account for the local formation of immune-complex deposits containing anionic antigens. For example, cationized IgG molecules can localize in glomeruli just as other cationic proteins do [65], and antibody IgG then can bind anionic antigen and thus initiate immune-complex formation locally [66]. The capacity of naturally occurring cationic IgG to localize in this way is suggested by studies demonstrating local formation of immune complexes of native BSA (pI 4.5) and anti-BSA antibody following several alternate perfusions of separated solutions of antigen and antibody in rat kidneys [67]. Subsequent studies demonstrating that immune deposits in this system form largely when the cationic antibody fraction is used support that hypothesis (FLEUREN G, et al, personal communication). Anionic antigens perhaps facilitate synthesis of such cationic antibody [68]. Experimental studies have demonstrated facilitated deposition of cationic antibody to both fixed and planted subepithelial antigens [69, 70], and elution studies have suggested a predominance of cationic antibodies in some naturally occurring models of glomerulonephritis [71, 72]. But elution studies must be interpreted with particular caution, as only a fraction of the deposited antibody is eluted, and that fraction has not been shown to be representative of the total antibody deposited.

A second mechanism of considerable potential importance with respect to anionic antigen localization involves initial interaction between nonimmune cationic proteins derived from inflammatory cells and platelets and glomerular anionic sites. Anionic antigens may then bind electrical y to deposited cationic proteins and initiate in-situ immune-complex formation. Several cationic proteins might participate in this process, including neutrophil cationic proteins [73], cationic products of complement activation [74], and platelet factor IV [75]. Acetyl glyceryl ether of phosphorylcholine (platelet activating factor, PAF), released from several inflammatory cells during immune-complex disease, may have similar effects by inducing release of platelet cationic proteins and vasoactive amines, which increase glomerular permeability and favor anionic antigen localization [76]. Immune-complex formation within the renal microvasculature can result in glomerular localization of platelet factor IV and platelet cationic proteins [77]. Enhanced glomerular immune-complex formation with anionic antigens in the presence of reduced capillary wall negative charge has been demonstrated with the polycation polyethyleneimmine (PEI). This polycation increases formation of mesangial, subendothelial and subepithelial immune-complex deposits induced by injection of anionic ferritin followed by antibody to it or by injection of native BSA-containing immune complexes [78, 79]. Subepithelial immune-complex deposits have been produced experimentally by the injection of cationized non-antibody IgG followed by anionic BSA and then anti-BSA antibody [80]. Evidence for a loss of glomerular anionic sites prior to the development of detectable glomerular immune-complex deposits in murine lupus nephritis suggests that such a process also might be operative in vivo [81, 82].

Two anionic antigens, DNA (pI 4.5) and a purified tubular brush-border antigen (pI 5.4), have been shown to bind directly to glomeruli, apparently by charge-independent mechanisms that have not been well defined [36, 83]. Antigen also may be localized in a subepithelial distribution by immunologic mechanisms in the form of IgG antibody to Heymann antigen (84, 85]. Finally, for immune-complex deposits formed by any of these mechanisms to persist and cause disease at any site, the deposits must be composed of precipitating antigen-antibody systems and be capable of undergoing rearrangement or condensation into large lattice-like aggregates that can be visualized by conventional immunofluorescence or electron microscopy [51, 86].

Circulating immune-complex trapping

The issue of whether preformed immune complexes can be passively trapped in a subepithelial site remains controversial, although I believe that this has not been convincingly shown to occur. In studies reporting subepithelial localization of complexes made of low-avidity antibodies [87] or non-covalently linked immune complexes made with cationic antigens [65], intravascular dissociation of antigen and antibody with subsequent in-situ immune-complex formation are likely to have occurred [88]. Subepithelial localization of covalently linked cationic immune complexes has been achieved in only one study, which did not define the size of the complexes deposited or carefully exclude small amounts of free cationic antigen. Again, local complex formation is a possible explanation for the results observed [89]. Charge neutralization has been suggested to facilitate subepithelial localization of preformed immune complexes [79].

Mesangial and subendothelial immune-complex deposits

Subendothelial deposits are not seen in the absence of mesangial deposits, and deposits at the two sites presumably develop by very similar mechanisms (Table 2). Diseases due to mesangial and subendothelial immune-complex deposits include lupus and type-I MPGN as well as a variety of postinfectious processes such as bacterial endocarditis, shunt nephritis, and diseases such as that of today's patient [6]. Deposits at mesangial and subendothelial sites can develop by in-situ mechanisms and from circulating immune-complex trapping.

In-situ immune-complex formation

Antibody binding to fixed glomerular antigens. Little clinical or experimental evidence currently exists for a fixed-antigen mechanism in the development of glomerulonephritis associated with mesangial deposits. However, glomerulonephritis has been produced with antibody to angiotensin converting enzyme, an antigen induced experimentally on the plasma membrane of endothelial cells [90]. Antibodies to endothelial cell-surface and Ia antigens have been reported in lupus and could contribute to the production of subendothelial deposits and glomerulonephritis by this mechanism [91, 92].

Planted non-glomerular antigens (Table 2). Antigen localization in a subendothelial distribution induces in-situ immune-complex formation and glomerulonephritis by two mechanisms. Large cationic antigens such as ferritin, which cannot readily penetrate the GBM, may localize by charge interaction with anionic sites on the inner surface of the capillary wall and produce subendothelial deposits [63]. The affinity of certain plant lectins, such as concanavalin A, for glucose and mannose residues in capillary wall glycoproteins also can result in glomerular localization of antigen. Injection of antibody to

"planted" concanavalin A results in a linear-granular pattern of subendothelial immune-complex deposits and glomerulonephritis [93]. Lectin-like components in some viruses may have the potential for producing deposits by such a mechanism in humans [43].

The mesangium is an intracapillary network of mesangial cells and matrix which, like the subendothelial space, is contiguous with the circulation through a layer of endothelial cells with fenestrae of about 44 nm [94]. It is therefore a ready repository for a variety of circulating macromolecules including potential antigens and preformed immune complexes. These molecules enter the mesangial matrix and are degraded locally by infiltrating monocytes or intrinsic mesangial cells, or they exit via the glomerular hilus into the cortical interstitium and renal lymph [95, 96]. This capacity of the mesangium to localize antigens, and the nephritogenicity of in-situ immune-complex formation within the mesangium, are well illustrated by the studies of Mauer and coworkers [61, 97]. Like many macromolecules, heat-aggregated human IgG administered intravenously into rabbits localizes within the mesangium. To exclude the presence of circulating antigen, rabbit kidneys containing the IgG mesangial deposits then were transplanted into normal rabbits before antibody to human IgG was administered. The resultant production of IgG–anti-IgG immune complexes within the mesangial matrix induced a focal proliferative glomerulonephritis much like that seen in several human renal diseases associated with mesangial immune-complex deposits, such as class II-III lupus nephritis, IgA nephropathy, and Henoch-Schönlein purpura [97]. In human IgA nephropathy, the IgA deposits appear to represent polymeric IgA antibodies of mucosal origin directed against some as yet unidentified antigen [98, 99]. Anionic charge sites have been identified in the mesangium [100, 101] and may play a role in the localization and persistence of cationic antigens, although this effect is much less evident than it is with capillary wall deposits [102].

Because subendothelial and mesangial deposits, whether formed locally or from circulating complex trapping, are in direct contact with immune reactants in the circulation, accretion of deposits with further glomerular injury may continue by other in-situ mechanisms, including the binding of rheumatoid factors [103] or antiidiotypic antibodies [104] to deposited IgG. Immune interaction also continues between reactive antigen or antibody binding sites in the deposits and their counterparts in immune complexes formed in the circulation [105].

Circulating immune-complex trapping

Numerous studies have demonstrated the capacity of preformed immune complexes injected intravenously to be passively trapped at mesangial and subendothelial sites (reviewed in Ref. 19). In contrast to the nephritis produced consistently when immune-complex deposits form in situ, however, the passive trapping of preformed complexes produces an influx of scavenging mononuclear cells [106] but little other histologic or functional evidence of glomerular disease [19]. More prolonged deposition of preformed complexes could be more productive of tissue damage. Until this phenomenon is demonstrated, however, the contention that glomerulonephritis can result from the passive trapping of preformed immune complexes must remain a hypothesis.

The factors regulating the glomerular deposition of preformed complexes from the circulation have been extensively studied and defined and are summarized in Table 2. They include systemic factors, glomerular factors, and properties of the immune complexes themselves. Systemic factors include renal blood flow, MPS function, and erythrocyte CR1 receptors, which collectively determine the plasma level, disappearance kinetics, and renal delivery of immune complexes [107–110]. Glomerular factors include hydrostatic pressure and filtration fraction, which determine the driving forces by which immune complexes enter the capillary wall or mesangium from the circulation [111], charge and permeability characteristics of the glomerulus itself [78, 79], and mesangial afferent and efferent limb or clearing function [61, 96, 112]. Properties of the immune complexes themselves are also important, particularly size and the determinants of size such as concentration of antigen and antibody, and antigen:antibody ratio, antigen valence, and antibody class and avidity [113]. Other properties of immune complexes such as charge, complement-fixing ability, and relative biodegradability also influence immune-complex deposition and persistence in glomeruli [89, 95, 109, 113, 114].

These same factors also influence the passive trapping of immune complexes in a subendothelial distribution. Subendothelial as well as mesangial deposits are seen when increased quantities of large immune complexes are delivered to the glomerulus, as can occur with a reduction in MPS capacity or mesangial clearing function [107, 108] or, in primates, perhaps by saturation of erythrocyte C3b (CR1) receptor function [109]. The largest and most persistent subendothelial deposits in experimental systems have been produced with large latticed preformed complexes made with cationized antibodies [114]. Alterations in capillary wall charge and permeability not only increase deposition but alter the pattern of immune-complex deposits and may facilitate subendothelial localization of preformed complexes [78, 79]. This finding may reflect changes in the size-selective glomerular filtration barrier that accompany charge reduction [115] or the increase in circulating immune complex size that results from loss of free antigen in the urine.

Finally, with respect to the mechanisms of glomerular immune-complex formation seen in response to exogenous antigens such as infectious agents, I must reemphasize the dynamic relationship always operative between antigen and antibody in free and immune-complex form ($Ag + Ab \rightleftarrows AgAb$). The status of this equilibrium is determined by the quantities of antigen and antibody available for immune-complex formation, antigen valence, the relative avidity of antibody for antigen, and the rate of clearance of the immune complexes formed. Thus, factors that reduce circulating immune-complex levels, such as enhanced MPS function or plasma-exchange therapy, also reduce levels of free antigen and antibody that may initiate in-situ immune-complex formation. Conversely, factors that increase circulating immune-complex levels, such as MPS blockade, also increase free circulating antigen and antibody levels. This increment in available reactants would enhance both circulating immune-complex deposition and, if appropriate conditions existed, in-situ immune-complex formation [116]. Thus, circulating immune complexes must be present, as they were in the patient we are discussing, for deposits to form from exogenous antigens by either immune-complex trapping or in-situ mechanisms. Circulating complexes provide a reservoir for the reactants that produce in-situ immune-complex deposits.

Measurement of serum levels or glomerular deposits of antigen, antibody, and immune complexes therefore cannot distinguish between these two mechanisms of deposit formation in either human or animal models such as serum sickness [62, 116].

Mediation of immune-complex-induced glomerular injury

The formation of antigen-antibody complexes within the glomerulus does not directly induce tissue injury. Rather, damage occurs as a consequence of activation of other cellular and humoral mediator systems. Because of the difficulty in producing experimental glomerular injury by deposition of preformed immune complexes, most studies of the mediation of immune renal injury have been carried out in models of in-situ immune-complex formation [28]. As I have mentioned, the mechanism by which immune-complex deposits form largely determines the intraglomerular site of deposit formation (Fig. 1). In turn, the type of inflammatory mediators activated (Fig. 2), and consequently the lesion produced, are importantly dependent on the site as well as on the composition of the immune deposits [117]. Four mechanisms have been established by which glomerular immune-complex deposits can initiate tissue injury; evidence is accumulating for a fifth. These mechanisms are illustrated schematically in Figure 2.

Direct effect of antibody deposition alone. The reaction of IgG antibody with GBM antigens can markedly increase glomerular permeability to protein independently of complement or inflammatory cells [118]. A similar effect has been demonstrated with univalent and divalent fragments of IgG antibody to the Heymann antigen on epithelial cell surfaces [119]. This effect occurs only with antibody to certain antigenic components of the glomerular capillary wall but not with others [120, 121]. The effect presumably reflects an internal alteration or distortion of the geometry of the filtration barrier and results in altered permselectivity. It has not been demonstrated with exogenous antigen-induced immune-complex formation in glomeruli.

Direct effect of complement. This newly recognized form of immune glomerular injury was first demonstrated in the passive Heymann nephritis model of membranous nephropathy induced by antibody reacting with a fixed subepithelial antigen. Proteinuria induced by in-situ subepithelial immune-complex formation was complement-dependent but did not involve inflammatory cells [122]. A similar mechanism is operative when the subepithelial deposits form in situ with planted exogenous antigens [84]. Studies of passive Heymann nephritis in rats made C6 deficient demonstrate a requirement for the C5b-9, or membrane attack complex (MAC), portion of the complement system for full development of proteinuria [123]. A similar requirement for C6 also has been demonstrated in cationic BSA-induced serum sickness [53] and in complement-dependent anti-GBM nephritis in the rabbit [124]. Further support for the membranolytic role of complement in immune glomerular injury is provided by the observation that neoantigens of the complement MAC are present, along with terminal complement components, in glomerular lesions induced by complement-dependent mechanisms but are absent in lesions in the genesis of which complement does not participate [125, 126]. The presence of neoantigens of the MAC in glomerular immune deposits in human renal diseases, including lupus nephritis and idiopathic membranous nephropathy [127, 128], suggests that a similar mechanism may be operative in humans. The molecular mechanism by which MAC formation and insertion into lipid bilayers of cell membranes—or perhaps into basement membranes [129]—alters the size-selective glomerular filtration barrier has not yet been defined.

Complement-neutrophil–mediated glomerular injury. The major mechanism of complement-mediated immune-complex–induced tissue injury has long been thought to be generation of chemotactic peptides, primarily C5a, by complement activation [130]. The generation of C5a results in neutrophil attraction to the site of immune deposits and, as a consequence, tissue injury is produced by release of toxic products of neutrophil activation adjacent to the GBM [20, 131, 132]. However, this mechanism has been demonstrated only in certain models of anti-GBM disease. Several observations document the importance of neutrophils in these models. Neutrophils invade glomeruli 4 to 24 hours following anti-GBM antibody deposition in quantities proportional to the amount of antibody deposited [131]. The onset of nonselective proteinuria correlates with the appearance of neutrophils, and the amount of proteinuria with their number [132, 133]. Proteinuria can be diminished or abolished by both complement and neutrophil depletion in some models [131, 132], and it can be produced by infusing neutrophils into neutrophil-depleted animals with antibody and complement deposits [134]. The importance of complement and C5a generation in neutrophil-mediated disease has been inferred from the beneficial effects of complement depletion and from the in-vitro chemotactic properties of C5a [135]. But as I have already mentioned, it now appears that a substantial portion of the complement effect may involve the terminal complement system [53, 123–126]. As shown in Figure 2, neutrophils also might be attracted through immune-adherence mechanisms involving C3b receptors [136] or might be attracted independently of complement via Fc receptor interaction with deposited immunoglobulins [137, 138].

The mechanism of neutrophil-mediated injury has been presumed to involve proteolytic digestion of GBM by enzymes released locally by invading neutrophils. This hypothesis is supported by *(1)* the ability of neutrophil-derived proteases to digest GBM in vitro [139, 140]; *(2)* the appearance of neutrophil-derived enzymes and GBM fragments in the urine in complement-dependent glomerular injury [139–141]; and *(3)* the presence of neutrophil cationic proteins in glomerular deposits [73]. More recent studies have suggested a role for reactive oxygen species (ROS) in neutrophil-mediated tissue injury [142, 143]. These ROS include hydrogen peroxide (H_2O_2), superoxide anion (O_2), hydroxyl radical ($\cdot OH$), and singlet oxygen($^1O_2$) released during the respiratory burst by activated phagocytic cells such as neutrophils and macrophages. Intrarenal infusion of stimulants to ROS generation produces a glomerular neutrophil infiltrate and proteinuria, which can be prevented by neutrophil depletion or by catalase, which destroys H_2O_2 [144]. Proteinuria in complement-neutrophil–dependent anti-GBM nephritis also can be reduced by catalase [145]. Presumably similar mechanisms are operative in macrophage-mediated glomerular injury.

Complement-independent, cell-mediated glomerular injury. Again, neutrophils appear to be able to mediate glomerular injury independently of complement when Fc portions of immune deposits are accessible for initiating immune adherence

[137, 138]. This action probably would apply with subendothelial and mesangial immune-complex deposits but is not seen with subepithelial deposits, which are separated from circulating inflammatory cells by a layer of GBM [84, 122]. Macrophages also participate in immune-complex disease through complement-independent mechanisms (Fig. 2). They are prominent in glomeruli in several models of immune-complex nephritis [146, 147] as well as in human disease [148, 149]. In addition to immune-adherence mechanisms, several platelet-derived cationic proteins that can localize in glomeruli and facilitate immune-complex formation also have chemotactic properties for inflammatory cells (reviewed in Ref. 150). When immune complexes form locally within the GBM, macrophage infiltrates and proteinuria can be much reduced by prior irradiation or by selective macrophage depletion [151, 152], and can be induced in cell-depleted animals by macrophages [153]. Macrophage depletion also reduces proteinuria in accelerated, acute BSA serum sickness [152]. The fact that this form of glomerular injury is ameliorated when induced by antibody lacking the Fc portion suggests that macrophages are recruited primarily by immune-adherence mechanisms [154]. Other studies have suggested, however, that macrophages follow an earlier T-cell infiltrate in glomeruli and that macrophage accumulation is reduced by T-cell depletion. The possibility thus exists of a sensitized cell-mediated phenomenon [155]. One study has suggested a role for non-sensitized lymphocytes, perhaps Fc-receptor–bearing natural killer cells, in mediating a focal loss of glomerular polyanion and proteinuria in early anti-GBM nephritis [156]. This observation may represent an antibody-dependent, cell-mediated cytotoxicity mechanism, which has been well documented in experimental antitubular basement membrane antibody-induced interstitial nephritis [157].

Finally, resident glomerular mesangial cells may participate in some types of immune glomerular disease [95, 158]. Mesangial cells thus can produce a variety of potential inflammatory mediators, including prostaglandins [159], acidic and neutral proteases [160, 161], ROS [162], and an interleukin 1-like cytokine [163]. Stimulants for such mesangial reactivity can include exposure to, or endocytosis of, several circulating or deposited immune reactants, including immune complexes, complement components, and platelet activating factor [164, 165]. The identification of a subpopulation of mesangial cells that bear Ia antigens, and which thus may present foreign antigens in a genetically restricted fashion to sensitized T cells, suggests that local cell-mediated immune reactors may occur in the mesangium [166]. Whether these various properties of the mesangial cell are pathogenetic in glomerular disease is not yet established.

Specifically sensitized cells. The role of antibody-independent cellular hypersensitivity to fixed or planted antigenic components of glomerular immune complexes in human disease has long been speculated on [167, 168] but usually has been discounted [169]. Experimental support for this mechanism is now mounting, however. Infusion of cells sensitized to an antigen planted in the glomerulus can induce glomerular hypercellularity [170] and sometimes proteinuria [171]. Bolton and coworkers induced glomerulonephritis by using GBM to immunize bursectomized chickens unable to mount an antibody response [172]. The authors reported transfer of this lesion with cells alone [173], and thus provided strong evidence for the capacity of sensitized cells to cause glomerular disease in that species. Thus, the cellular arm of the immune response to antigens that induce immune-complex nephritis may play a previously unappreciated role in producing these lesions.

Summary

The patient we are discussing here had an acute glomerulonephritis induced by immune-complex formation in glomeruli. The lesion apparently was related to a chronic bacterial infection. Glomerulonephritis resulted from immune-complex deposits formed at mesangial, subendothelial, and subepithelial sites. Removal of the offending antigenic stimulus resulted in clinical improvement and presumably resolution of the immune-complex deposits. It should be apparent that the type and severity of her glomerular disease were determined by at least four factors: *(1): The mechanism of glomerular immune-complex formation.* For example, formation of immune complexes in situ in the mesangium results in a severe focal proliferative glomerulonephritis, whereas deposition of apparently similar quantities of the same reactants as preformed immune complexes induces little evidence of glomerular disease [19, 97]. *(2) The intraglomerular site of immune-complex formation.* Immune complexes formed in situ in the subepithelial space produce a noninflammatory, complement-dependent, cell-independent membranous glomerular lesion [84, 122], whereas the formation of the same quantity of the same complexes within the capillary wall, and therefore more accessible to circulating inflammatory cells, induces a proliferative inflammatory lesion that is complement and neutrophil or macrophage mediated [117, 137, 152]. *(3) The biologic properties of the deposited antibody.* Subepithelial deposits of complement-fixing IgG result in a marked complement-dependent increase in glomerular capillary permeability, whereas deposition of the same quantity of non–complement-fixing antibody at the same site produces no detectable glomerular injury [122]. *(4) Finally, if other factors are equal, the greater the quantity of deposits formed, the more severe the disease produced* [32, 131].

Obviously, considerable progress has been made recently in our understanding of the pathogenesis of immune-complex glomerulonephritis like this patient's. But only by further study of these mechanisms can we hope to continue to advance our understanding of human immune-complex nephritis and thereby acquire the capacity to modify it in a way that will be beneficial to patients such as the one discussed here.

Questions and answers

DR. CHRISTINE ABRASS *(Associate Professor of Medicine, Division of Nephrology, University of Washington, Seattle Veterans Administration Hospital):* You have reviewed thoroughly the data that support the in-situ mechanism, and I think there is little doubt that this mechanism plays an important role in immune-complex nephritis. On the other hand, over the last several years it has become a bandwagon that many investigators have jumped on, and experiments have been contrived to prove its importance. Few investigators have designed experiments using modern techniques to support the circulating immune-complex mechanism. We were relatively narrow-minded for many years, accepting only the circulating immune-complex theory. More recently, we are told to believe only the in-situ theory. It may be more appropriate to consider that both

of these mechanisms are operative in immune-complex nephritis.

DR. COUSER: This is a very important point. Bandwagons clearly develop in medicine. The relatively uncritical acceptance of the circulating immune-complex trapping mechanism is an example that lasted for more than two decades. Now we risk a similar phenomenon with respect to in-situ complex formation. Obviously, it is important that we avoid overinterpretation of these recent studies as well. However, I also think that the experimental evidence that in-situ immune complex formation can reproduce the clinical and histologic features of most forms of immune-complex nephritis in humans has now become very strong [28, 174]. The contention that similar lesions can result from circulating complex trapping remains unproved, although it was suggested more than 25 years ago [16, 17]. I agree, however, that more studies in this area are needed and that it is critical that objectivity be retained when we interpret the data that emerge.

DR. REX OCHI *(Nephrology Fellow, University of Washington):* In patients with glomerulonephritis, what is the clinical role for measuring circulating immune complexes?

DR. COUSER: That is a good question. Obviously development of techniques for measuring circulating immune complexes was stimulated by the thesis that they were the causative agents in these diseases and from expectations that such measurements would be of diagnostic and prognostic value. With the exception of one study in lupus by Abrass et al [175], these expectations have generally not been fulfilled [176, 177]. In my view, the measurement of circulating immune complexes is of no particular value in any renal disease that I am aware of. I include lupus unless the measurements can be done with the same frequency and intensity as they were done by Dr. Abrass in her study [175]. However, as I discussed earlier, that does not mean that the reactants that form glomerular deposits are not present in circulating immune-complex form, but only that the variables that lead to immune-deposit formation in glomeruli are not reflected in a clinically useful way by measurements of total circulating immune-complex levels.

DR. STEVEN ADLER *(Assistant Professor of Medicine, Division of Nephrology, University of Washington, University Hospital):* Immunologic mechanisms obviously are important in most of the acute glomerular diseases we see, but do these mechanisms have a role in the progression of renal disease?

DR. COUSER: For several decades investigators have sought immunologic processes such as cellular immunity to renal antigens to account for progressive glomerulonephritis. I think all this work can be summarized simply by saying that none have been reproducibly found. As you point out, the immunologic mechanisms I have discussed do account well for the initiation of most forms of glomerulonephritis and, if severe enough, lead to progressive disease. But they do not explain progression. Although the thesis has not been proved in humans, I think the studies by Brenner and colleagues in rats have established adaptive hemodynamic factors, particularly increased intraglomerular pressures, as the most likely mechanisms responsible for progressive glomerular disease [178]. Presumably these adaptive changes in glomerular pressures and flows must exceed a threshold level to cause progressive disease, and in turn a critical amount of filtering surface area must be irreversibly lost during the acute phase of the disease for this to occur. Humans might be less susceptible to these changes than are rats, and other factors are likely to be important as well. However, there is little evidence at present to implicate immune mechanisms in this process.

DR. JEROME P. KASSIRER: It always has been puzzling to me that some forms of immune-complex disease are spontaneously reversible and others are not. For example, in poststreptococcal glomerulonephritis the patient has a severe immune reaction accompanied by a severe reduction in GFR, yet recovery is often rapid and complete. By contrast, the patient with lupus who also has severe immunologic damage usually doesn't get better even with aggressive therapy. What is the explanation for this difference?

DR. COUSER: I think lupus is probably not a good example because in lupus there is an ongoing autoimmune process, whereas in poststreptococcal nephritis the disease is usually self-limited even without therapy. Recovery depends on how much the initial decrease in renal function reflects reversible, or functional, factors rather than irreversible structural changes such as necrosis, sclerosis, and fibrosis. Postinfectious glomerulonephritis may be quite analogous to models of acute nephritis studied experimentally, in which the decreased GFR is consequent to changes in glomerular plasma flow and ultrafiltration coefficient (Kf) apparently reflecting hemodynamic consequences of complement activation and loss of surface area due to inflammatory cell infiltrates [179, 180]. Because these are largely functional changes, they may be reversed spontaneously or by appropriate therapy before structural damage occurs. In other forms of acute glomerulonephritis, the initial process may be severe enough to produce irreversible structural damage so that recovery is not possible, or it occurs only accompanied by adaptive hemodynamic changes sufficient to result in progressive disease [181]. Reversibility depends on the type and severity of the initial lesion rather than on the mechanism that produced it.

DR. RICHARD JOHNSON *(Nephrology Fellow, University of Washington):* Are there any factors that might predispose certain individuals over others to the development of postinfectious glomerulonephritis?

DR. COUSER: Yes, there clearly are. For example, in large populations of patients exposed to the same nephritogenic streptococcus, some patients have no disease, some have subclinical disease, and some have severe glomerulonephritis [182]. Some of this variation must relate to the amount and duration of antigenic exposure. However, much of the variation is also related to host genetic factors that determine the immune response to a particular antigen [183]. The influence of immunogenetic factors on severity and prognosis has been well established by familial and genetic studies for several glomerular diseases [183–185]. In poststreptococcal glomerulonephritis, associations have been noted with HLA-D and -DR antigens in some studies [186, 187]. Although it may be difficult and expensive to carry out, the importance of immunogenetic factors to the severity and outcome of glomerulonephritis is now sufficiently well established, so this variable should be accounted for in designing randomized prospective treatment studies.

DR. MICHAEL R. KELLY *(Clinical Professor of Medicine, University of Washington, Swedish Hospital):* In clinical nephrology, we usually regard the C3 level as important in

helping to make a diagnosis of postinfectious glomerulonephritis. In this patient, complement levels were normal. Would you comment on the value of C3 measurements in these kinds of patients?

DR. COUSER: In classic poststreptococcal glomerulonephritis, levels of complement as measured by C3, or total hemolytic complement, are reduced acutely in approximately 90% of patients, so these measurements are relatively sensitive in making a diagnosis [188–190]. However, cases of apparently clinically typical acute poststreptococcal nephritis with normal complement levels have been described [191, 192]. The presence of hypocomplementemia is obviously not specific for poststreptococcal nephritis, because it also can be seen in other postinfectious nephritides, shunt nephritis, membranoproliferative glomerulonephritis, lupus, and certain inherited complement deficiencies associated with nephritis [190]. In glomerulonephritis following nonstreptococcal bacterial infections such as that probably in this patient, hypocomplementemia is less common. It is present in only approximately 50% of patients with nephritis due to bacterial endocarditis [193], and often is absent in nephritis associated with visceral abscesses [194]. Serum complement levels must be interpreted in the context of the rest of the clinical and laboratory findings.

DR. DAVID LOVETT *(Assistant Professor of Medicine, Division of Nephrology, University of Washington, Seattle Veterans Administration Hospital):* You have emphasized the role of immune complexes formed in situ in initiating complement activation. Is there evidence for nonimmune local complement activation in the absence of immune deposits, and what role might such a process play in glomerular disease?

DR. COUSER: Yes, there are examples of glomerular disease such as type-II membranoproliferative glumerulonephritis, late stages of postinfectious nephritis, and others in which deposits of complement components can be seen in the absence of immune deposits [5, 196]. Furthermore, complement components and membrane attack complex neoantigens are often associated with structural glomerular lesions such as sclerosis, fibrin caps, and PAS-positive deposits in diabetes, etc. [128]. Some of these deposits might be epiphenomena reflecting complement activation by damaged kidney cells, as we have shown in vitro [197]. However, there is now substantial evidence that the C5b-9 portion of complement can have a nonlytic effect on cell membranes and can result in increased production of several inflammatory mediators [198]. Your own studies and those of Dr. Adler have shown increased production of proteases, prostanoids, interleukin 1-like cytokines, and reactive oxygen species by mesangial cells in response to C5b-9 without immune deposits [165, 199]. The potential role of resident glomerular cells in mediating glomerular disease and of complement in stimulating that process is obviously a fruitful area for future research.

DR. KASSIRER: Do you think it will be possible to tailor our treatment to the specific immunologic mechanism responsible for glomerular injury?

DR. COUSER: Yes, I do. Unfortunately, patients do not come to our attention until they already have significant disease, so prevention is difficult, although much progress is being made in reducing the incidence of poststreptococcal glomerulonephritis in this country. However, as I mentioned earlier, a critical determinant of outcome in these diseases is how successful we can be in preventing irreversible tissue damage during the acute phase of the disease. Clarification of the mechanisms involved in mediating specific types of glomerular immune injury such as terminal complement components, macrophages, sensitized cells, reactive oxygen species, etc. does afford the potential for directed therapeutic interventions that may minimize or prevent further tissue damage once an accurate diagnosis has been made.

DR. THERESA RATTAZZI *(Clinical Assistant Professor of Medicine, University of Washington, Valley General Hospital):* If immunologic mechanisms play a role in the initiation of glomerulonephritis but not in the progression of the disease, what is the role of steroids in treating glomerular disease and why do we use them?

DR. COUSER: The answer to why we use steroids probably derives largely from their dramatic effect in minimal-change nephrotic syndrome and the hope, which has largely been unfulfilled, that they would be similarly beneficial in other glomerular diseases, which are now known to be mediated by entirely different mechanisms. However, steroids do appear to be of benefit in several acute inflammatory forms of glomerulonephritis such as lupus nephritis [200] and idiopathic, rapidly progressive glomerulonephritis [201]. The mechanism for their beneficial effects in these diseases is unknown, although the accumulating evidence for a role for cell-mediated immunity in glomerulonephritis, which I have already reviewed, is one attractive possibility. A short-term course of steroids also appears to be beneficial in slowing the rate of progression of certain chronic glomerular diseases such as membranous nephropathy [202] and perhaps focal glomerular sclerosis (Unpublished data, Collaborative study of adult glomerular disease, C. H. Coggins, Director). In these diseases the mechanism of the steroid effect is even less clear but may be on nonimmune factors that influence progression rather than on immune factors that initiated the diseases.

Reprint requests to Dr. W. Couser, Division of Nephrology, RM-11, School of Medicine, University of Washington, Seattle, Washington 98195, USA

References

1. CUNNINGHAM EE, BRENTJENS JR, ZIELEZNY MA, ANDRES GA, VENUTO RC: Heroin nephropathy. A clinicopathologic and epidemiologic study. *Am J Med* 68:47–53, 1980
2. RAO TKS, NICASTRI AD, FRIEDMAN EA: Renal consequences of narcotic abuse. *Adv Nephrol* 7:261–290, 1978
3. CITRON PB, HALPERN M, MCCARRON M, LUNDBERG GD, MCCORMICK R, PINCUS IJ, TATTER D, HAVERBACK BJ: Necrotizing angiitis associated with drug abuse. *N Engl J Med* 283:1001–1011, 1970
4. STEINMULLER DR, BOLTON WK, STILMANT MM, COUSER WG: Chronic interstitial nephritis and mixed cryoglobulinemia associated with drug abuse. *Arch Pathol Lab Med* 103:63–66, 1979
5. MENSCHEL S, COHEN D, GROSS E, FRANGIONE B, GALLO G: AA protein-related renal amyloidosis in drug addicts. *Am J Pathol* 112:195–199, 1983
6. COUSER WG: Glomerular diseases (chapter 62), in *Cecil's Textbook of Medicine* (17th ed), edited by WYNGAARDEN JB, SMITH LH JR, Philadelphia, Saunders, 1985, pp 568–589
7. BARNHAM M, THORNTON TJ, LANGE K: Nephritis caused by *Streptococcus zooepidemicus* (Lancefield group C). *Lancet* 1:945–948, 1983
8. SATO M, NAKAZORA H, OFUJI T: The pathogenetic role of *Staphylococcus aureus* in primary human glomerulonephritis. *Clin Nephrol* 11:190–195, 1979

9. BOLTON WK, SANDE AM, NORMANSELL DE, STURGILL BC, WESTERVELT FB JR: Ventriculojugular shunt nephritis with *Corynebacterium bovis*. Successful therapy with antibiotics. *Am J Med* 59:417–424, 1975
10. LOHLEIN M: Über die entzündlichen Veränderungen der Glomeruli der menschlichen Nieren und ihre Bedeutung für die Nephritis. Arbeiten aus dem Pathologischen Institut zu Leipzig. Leipzig, S. Hirzel, 1907
11. SCHICK B: Die Nachkrankheiten des Scharlachs. *Jahrb Kinderheil* 65:132–173, 1907
12. VON PIRQUET CE: Allergy. *Arch Intern Med* 7:259–288, 383–436, 1911
13. MELLORS RC, ORTEGA LG: Analytical pathology: III. New observations on the pathogenesis of glomerulonephritis, lipoid nephrosis, polyarteritis nodosa and secondary amyloidosis in man. *Am J Pathol* 32:455–499, 1956
14. GERMUTH GF JR, PACE MG, TIPPETT JC: Comparative histologic and immunologic studies in rabbits of induced hypersensitivity of the serum sickness type: II. The effect of sensitization to homologous and cross-reactive antigens on the rate of antigen elimination and the development of allergic lesions. *J Exp Med* 101:135–150, 1955
15. GERMUTH FG JR, FLANAGAN C, MONTIENEGRO MR: The relationships between the chemical nature of the antigen, antigen dosage, rate of antibody synthesis and the occurrence of arteritis and glomerulonephritis in experimental hypersensitivity. *Johns Hopkins Med J* 101:149–169, 1957
16. DIXON FJ, VAZQUEZ JJ, WEIGEL WO, COCHRANE CG: Pathogenesis of serum sickness. *Arch Pathol* 68:18–28, 1958
17. DIXON FJ, FELDMAN JD, VAZQUEZ JJ: Experimental glomerulonephritis: The pathogenesis of a laboratory model resembling the spectrum of human glomerulonephritis. *J Exp Med* 113:899–920, 1961
18. GERMUTH FG JR, RODRIGUEZ E: in *Immunopathology of the Renal Glomerulus: Immune Complex Deposit and Anti-basement Membrane Disease*. Boston, Little, Brown, 1973
19. COUSER WG, SALANT DJ: In-situ immune complex formation and glomerular injury *(editorial review)*. *Kidney Int* 17:1–13, 1980
20. COCHRANE CG: Mediation of immunologic glomerular injury. *Transplant Proc* 1:949–958, 1969
21. COUSER WG, HOYER JR, JERMANOVICH NB, BELOK S, STILMANT MM: Effect of aminonucleoside nephrosis on immune complex localization in autologous immune complex nephropathy in rats. *J Clin Invest* 61:561–572, 1978
22. COUSER WG, SALANT DJ, STILMANT MM, ARBEIT LA, DARBY C, SLIOGERIS VS: The effects of aminonucleoside of puromycin and nephrotoxic serum on subepithelial immune-deposit formation in passive Heymann nephritis. *J Lab Clin Med* 94:917–932, 1979
23. SALANT DJ, BELOK S, DARBY C, STILMANT MM, COUSER WG: Determinants of glomerular localization of subepithelial immune deposits: Effects of altered antigen:antibody ratio, steroids, vasoactive amine antagonists and aminonucleoside of puromycin on passive Heymann nephritis in rats. *Lab Invest* 41:89–99, 1979
24. EDINGTON TS, GLASSOCK RJ, DIXON FJ: Autologous immune complex pathogenesis of experimental allergic glomerulonephritis. *Science* 155:1432–1434, 1967
25. VAN DAMME BJC, FLEUREN GJ, BAKKER WW, VERNIER RL, HOEDEMAEKER PJ: Experimental glomerulonephritis in the rat induced by antibodies directed against tubular antigens: IV. Fixed glomerular antigens in the pathogenesis of heterologous immune complex glomerulonephritis. *Lab Invest* 38:502–510, 1978
26. COUSER WG, STEINMULLER DR, STILMANT MM, SALANT DJ, LOWENSTEIN LM: Experimental glomerulonephritis in the isolated perfused rat kidney. *J Clin Invest* 62:1275–1287, 1978
27. COUSER WG, ADLER SA, BAKER PJ, JOHNSON RJ, PERKINSON DA: Mechanisms of immune complex formation and deposition in glomeruli. *Nephrology Today: Proc 9th Int Cong Nephrol*. New York, Springer-Verlag, pp 508–526
28. COUSER WG, SALANT DJ: Immunopathogenesis of glomerular capillary wall injury in nephrotic states. *Contemp Issues Nephrol* 9:47–83, 1982
29. KERJASCHKI D, FARQUHAR MG: The pathogenic antigen of Heymann nephritis is a membrane glycoprotein of the renal proximal tubule brush border. *Proc Natl Acad Sci USA* 79:5557–5561, 1982
30. KERJASCHKI D, FARQUHAR MG: Immunocytochemical localization of the Heymann nephritis antigen (GP330) in glomerular epithelial cells of normal Lewis rats. *J Exp Med* 157:667–685, 1983
31. UNANUE ER, DIXON FJ: Experimental glomerulonephritis. V. Studies on the interaction of nephrotoxic antibodies with tissues of the rat. *J Exp Med* 121:697–714, 1965
32. SALANT DJ, DARBY C, COUSER WG: Experimental membranous glomerulonephritis in rats. Quantitative studies of glomerular immune deposit formation in isolated glomeruli and whole animals. *J Clin Invest* 66:71–81, 1980
33. BARBA LM, CALDWELL PRB, DOWNIE GH, CAMUSSI G, BRENTJENS JR, ANDRES GA: Lung injury mediated by antibodies to endothelium: I. In the rabbit repeated interaction of heterologous anti-angiotensin-converting enzyme antibodies with alveolar endothelium results in resistance to immune injury through antigenic modulation. *J Exp Med* 158:2141–2158, 1983
34. MATSUO S, CALDWELL PRB, BRENTJENS JR, ANDRES GA: "In vivo" interaction of antibodies with cell surface antigens: A mechanism responsible for "in situ" formation of immune deposits in the zona pellucida of rabbit oocytes. *J Clin Invest* 75:1369–1380, 1985
35. CAMUSSI G, BRENTJENS JR, KERJASCHKI D, MALAVASI F, NOBLE D, ROHOLT OA, FARQUHAR MG, ANDRES G: Antibody-induced redistribution of Heymann's antigen on the surface of visceral glomerular epithelial cells in Lewis rats *(abstract)*. *Kidney Int* 27:207, 1985
36. ABRASS CK: Direct binding of renal tubular antigen to the subepithelial space of the glomerulus *(abstract)*. *Abstr 9th Int Cong Nephrol*, Los Angeles, 1984, p 231a
37. ABRASS CK, MCVAY J, GLASSOCK RJ: Evaluation of homologous and isologous passive Heymann nephritis: Influence on endogenous antibody production. *J Immunol* 130:195–202, 1983
38. NEALE TJ, WOODROFFE AJ, WILSON CB: Spontaneous glomerulonephritis in rabbits. Role of a glomerular capillary antigen. *Kidney Int* 26:701–711, 1984
39. DOUGLAS MFS, RABIDEAU DP, SCHWARTZ MM, LEWIS EJ: Evidence of autologous immune-complex nephritis. *N Engl J Med* 305:1326–1329, 1981
40. ZANETTI M, MANDET C, DUBOUST A, BEDROSSIAN J, BARIETY J: Demonstration of a passive Heymann nephritis-like mechanism in a human kidney transplant. *Clin Nephrol* 15:272–277, 1981
41. ZAGER RA, COUSER WG, ANDREWS BS, BOLTON WK, POHL MA: Radioimmunologic search for anti-tubular epithelial antibodies and circulating immune complexes in patients with membranous nephropathy. *Nephron* 24:10–16, 1979
42. COLLINS AD, ANDRES GA, MCCLUSKEY RT: Lack of evidence for a role of renal tubular antigen in human membranous glomerulonephritis. *Nephron* 27:297–301, 1981
43. NEALE TJ, WILSON CB: Glomerular antigens and glomerulonephritis. *Springer Semin Immunopathol* 5:221–249, 1982
44. ADLER S, COUSER WG: Immunologic mechanisms of renal disease *(edit rev)*. *Am J Med Sci* 289:55–60, 1985
45. SHOENFELD Y, RAUCH J, MASSICOTTE H, DATTA SK, ANDRED-SCHWARTZ J, STOLLAR BD, SCHWARTZ RS: Polyspecificity of monoclonal lupus autoantibodies produced by human-human hybridomas. *N Engl J Med* 308:414–420, 1983
46. MADAIO MP, CARLSON JA, HODDER S: Monoclonal anti-DNA antibodies bind directly to intrinsic glomerular antigens and form immune deposits *(abstract)*. *Kidney Int* 27:216a, 1985
47. BORDER WA, WARD HJ, KAMIL ES, COHEN AH: Induction of membranous nephropathy in rabbits by administration of an exogenous cationic antigen: Demonstration of a pathogenic role for electrical charge. *J Clin Invest* 69:451–461, 1982
48. OITE T, BATSFORD SR, MIHATSCH MJ, TAKAMIYA H, VOGT A: Quantitative studies of in situ immune complex glomerulonephritis in the rat induced by planted cationized antigen. *J Exp Med* 155:460–474, 1982
49. WARD HJ, COHEN AH, BORDER WA: In situ formation of subepithelial immune complexes in the rabbit glomerulus: Requirement of a cationic antigen. *Nephron* 36:257–264, 1984
50. TANAKA T: Gels. *Sci Am* 224:124–138, 1981
51. AGODOA LYC, GAUTHIER VJ, MANNIK M: Precipitating antigen

antibody systems are required for the formation of subepithelial electron dense immune deposits in rat glomeruli. *J Exp Med* 158:1259–1271, 1983
52. ADLER SG, WANG H, WARD HJ, COHEN AH, BORDER WA: Electrical charge: Its role in the pathogenesis and prevention of experimental membranous nephropathy in the rabbit. *J Clin Invest* 71:487–499, 1983
53. GROGGEL GC, ADLER S, RENNKE HG, COUSER WG, SALANT DJ: Role of the terminal complement pathway in experimental membranous nephropathy in the rabbit. *J Clin Invest* 72:1948–1957, 1983
54. ADLER S, BAKER P, PRITZL P, COUSER WG: Effect of alterations in glomerular charge on deposition of cationic and anionic antibodies to fixed glomerular antigens in the rat. *J Lab Clin Med*, in press
55. VOGT A, BATSFORD S, RODRIGUEZ-ITURBE B, GARCIA R: Cationic antigens in poststreptococcal glomerulonephritis. *Clin Nephrol* 20:271–279, 1983
56. LANGE K, SELIGSON G, CRONIN W: Evidence for the in situ origin of poststreptococcal glomerulonephritis: glomerular localization of endostreptosin and the clinical significance of the subsequent antibody response. *Clin Nephrol* 19:3–10, 1983
57. MCINTOSH RM, GARCIA R, RABIDEAU D, RODRIGUEZ-ITURBE B: Evidence for an autologous immune complex pathogenic mechanism in acute poststreptococcal glomerulonephritis. *Kidney Int* 14:501–510, 1978
58. RODRIGUEZ-ITURBE B, RABIDEAU D, GARCIA R, RUBIO L, MCINTOSH RM: Characterization of the glomerular antibody in acute poststreptococcal glomerulonephritis. *Ann Intern Med* 92:478–481, 1980
59. KAPLAN MH: Localization of streptococcal antigens in tissues. I. Histologic distribution and persistence of M protein types 1, 5, 12 and 19 in the tissues of the mouse. *J Exp Med* 107:341, 1958
60. MILLER F: Renal localization and persistence of type 12 streptococcal M-protein. *Proc Soc Exp Biol Med* 108:539–543, 1961
61. MAUER SM, FISH AJ, BLAU EB, MICHAEL AF: The glomerular mesangium: I. Kinetic studies of macromolecular uptake in normal and nephrotic rats. *J Clin Invest* 51:1092–1099, 1972
62. COUSER WG: What are circulating immune complexes doing in glomerulonephritis? *(editorial) N Engl J Med* 300:1230–1232, 1981
63. VOGT A, ROHRBACH R, SHIMIZU F, TAKAMIYA H, BATSFORD S: Interaction of cationized antigen with rat glomerular basement membrane: In situ immune complex formation. *Kidney Int* 22:27–35, 1982
64. VOGT A: New aspects of the pathogenesis of immune complex glomerulonephritis: Formation of subepithelial deposits. *Clin Nephrol* 21:15–20, 1984
65. GALLO GR, CAULIN-GLASER T, LAMM ME: Charge of circulating immune complexes as a factor in glomerular basement membrane localization in mice. *J Clin Invest* 67:1305–1313, 1981
66. AGODOA LYC, GAUTHIER VJ, MANNIK M: Antibody localization in the glomerular basement membrane may precede in situ immune deposit formation in rat glomeruli. *J Immunol*, 134:880–884, 1985
67. FLEUREN G, GROND J, HOEDEMAEKER PJ: In situ formation of subepithelial glomerular immune complexes in passive serum sickness. *Kidney Int* 17:631–637, 1980
68. SELA M, MOZES E: Dependence of the chemical nature of antibodies on the net electrical charge of antigens. *Proc Natl Acad Sci USA* 55:445–452, 1966
69. MADAIO MP, SALANT DJ, ADLER S, DARBY C, COUSER WG: Effect of antibody charge and concentration on glomerular deposition of anti-GBM antibody. *Kidney Int* 26:397–403, 1984
70. FEINTZEIG ID, DITTMER JE, SALANT DJ: The charge of antibody, antigen and the glomerular capillary wall and the location of antigen influence antibody deposition in experimental glomerulonephritis. *Kidney Int*, in press
71. EBLING F, HAHN BH: Restricted subpopulations of DNA antibodies in kidneys of mice with systemic lupus. Comparison of antibodies in serum and renal eluates. *Arth Rheum* 23:392–403, 1980
72. DANG H, HARBECK RJ: A comparison of anti-DNA antibodies from serum and kidney eluates of NZB x NZW F_1 mice. *J Clin Lab Immunol* 9:139–145, 1982
73. CAMUSSI G, TETTA C, SEGOLONI G, KODA R, VERCELLONE A: Localization of neutrophil cationic proteins and loss of anionic charges in glomeruli of patients with systemic lupus erythematosus. *Clin Immunol Immunopathol* 24:299–314, 1982
74. FERNANDEZ HM, HUGLI TE: Partial characterization of human C5a anaphylatoxin. I. Chemical description of the carbohydrate and polypeptide portion of C5a. *J Immunol* 117:1688–1694, 1976
75. BARNES JL, LEVINE PS, VENKATACHALAM MA: Binding of platelet factor four (PF4) to glomerular polyanion. *Kidney Int* 25:759–765, 1984
76. CAMUSSI G, TETTA C, CODA R, SOGOLONI GP, VERCELLONE A: Platelet-activating factor-induced loss of glomerular anionic charges. *Kidney Int* 25:73–81, 1984
77. BARNES JL, CAMUSSI G, TETTA C, VENKATACHALAM MA: Glomerular localization of platelet cationic proteins following immune complex induced platelet activation *(abstract)*. *Kidney Int* 27:206, 1985
78. BARNES JL, VENKATACHALAM MA: Glomerular interactions of exogenous and endogenous polycations, in *The Pathogenicity of Cationic Proteins*, edited by LAMBERT PP, BERGMANN P, BEAUWENS R, New York, Raven Press, 1983, pp 281–293
79. BARNES JL, VENKATACHALAM MA: Enhancement of glomerular immune complex deposition by circulating polycation. *J Exp Med* 160:286–293, 1984
80. CHAN EKL, BOYD ND, FRITZLER M: In vivo anionic immune complex deposition in the murine glomerulus *(abstract)*. *Clin Res* 31:448, 1983
81. MELNICK GF, LADOULIS CT, CAVALLO T: Decreased anionic groups and increased permeability precedes deposition of immune complexes in the glomerular capillary wall. *Am J Pathol* 105:114–120, 1981
82. CAVALLO T, GOLDMAN M, GRAVES K, LAMBERT P-H: Altered glomerular permeability in the early phase of immune complex nephritis. *Kidney Int* 24:632–637, 1983
83. CARLSON JA, MADAIO MP: Low molecular weight DNA binds to normal mouse glomeruli in vivo *(abstract)*. *Kidney Int* 25:210, 1984
84. VAN ES LA, BLOK APR, SCHOENFELD L, GLASSOCK RJ: Chronic nephritis induced by antibodies reacting with glomerular bound immune complexes. *Kidney Int* 11:106–115, 1977
85. ADLER S, SALANT DJ, DITTMER JE, RENNKE HG, MADAIO MP, COUSER WG: Mediation of proteinuria in membranous nephropathy due to a planted glomerular antigen. *Kidney Int* 23:807–815, 1983
86. MANNIK M, AGODOA LYC, DAVID KA: Rearrangement of immune complexes in glomeruli leads to persistence and development of electron dense deposits. *J Exp Med* 157:1516–1528, 1983
87. GERMUTH FG JR, RODRIGUEZ E, LORELLE CA, TRUMP EI, MILANO L, WISE O'L: Passive immune complex glomerulonephritis in mice: Models for various lesions found in human disease. II. Low avidity complexes and diffuse proliferative glomerulonephritis with subepithelial deposits. *Lab Invest* 41:366–371, 1979
88. GERMUTH FG JR, RODRIGUEZ E, WISE O'L: Passive immune complex glomerulonephritis in mice. III. Clearance kinetics and properties of circulating complexes. *Lab Invest* 46:515–519, 1982
89. CAULIN-GLASSER T, GALLO GR, LAMM ME: Nondissociating cationic immune complexes can deposit in glomerular basement membrane. *J Exp Med* 158:1561–1572, 1983
90. MATSUO S, CALDWELL P, BRENTJENS J, ANDRES G: Nephrotoxic serum glomerulonephritis induced in the rabbit by anti-endothelial antibodies *(abstract)*. *Kidney Int* 27:217, 1985
91. CINES DB, LYSS AP, REEBER MB, DEHORATIUS RJ: Presence of complement-fixing anti-endothelial cell antibodies in systemic lupus erythematosus. *J Clin Invest* 73:611–625, 1984
92. OKUDAIRA K, SEARLES RP, CEUPPENS JL, GOODWIN JS, WILLIAMS RC JR: Anti-Ia reactivity in sera from patients with systemic lupus erythematosus. *J Clin Invest* 69:17–24, 1982
93. GOLBUS SM, WILSON CB: Experimental glomerulonephritis induced by in situ formation of immune complexes in the glomerular capillary wall. *Kidney Int* 16:148–157, 1979
94. KANWAR YS: Biology of disease. Biophysiology of glomerular filtration and proteinuria. *Lab Invest* 51:7–21, 1984
95. STERZEL RB, LOVETT DH, STEIN HD, KASHGARIAN M: The

mesangium and glomerulonephritis. *Klin Wochenschr* 60:1077–1094, 1982
96. MICHAEL AF, KEANE WF, RAIJ L, VERNIER RL, MAUER SM: The glomerular mesangium (editorial review). *Kidney Int* 17:141–154, 1980
97. MAUER SM, SUTHERLAND DER, HOWARD RJ, FISH AJ, NAJARIAN JS, MICHAEL AF: The glomerular mesangium: III. Acute immune mesangial injury: A new model of glomerulonephritis. *J Exp Med* 137:553–570, 1973
98. TOMINO Y, ENDOH M, NOMOTO Y, SAKAI H: Specificity of eluted antibody from renal tissues of patients with IgA nephropathy. *Am J Kidney Dis* 1:276–280, 1982
99. COUSER WG: Mesangial IgA nephropathies: Steady progress (editorial). *West J Med* 140:89–91, 1984
100. KANWAR YS, JAKUBOWSKI ML, ROSENZWEIG LJ: Distribution of sulfated glycosaminoglycans in the glomerular basement membrane and mesangial matrix. *Eur J Cell Biol* 31:290–295, 1983
101. COHEN S, VERNIER RL, MICHAEL AF: The effect of charge on the renal distribution of ferritin. *Am J Pathol* 110:170–181, 1983
102. GAUTHIER VJ, STRIKER GE, MANNIK M: Glomerular localization of immune complexes prepared with anionic antibodies or with cationic antigen. *Lab Invest* 50:125–137, 1984
103. FORD PM, KOSATKA I: In situ immune complex formation in the mouse glomerulus: Reactivity with human IgM rheumatoid factor and the effect of subsequent immune complex deposition. *Clin Exp Immunol* 51:285–291, 1983
104. ZANETTI M, WILSON CB: Participation of auto-anti-idiotypes in immune complex glomerulonephritis in rabbits. *J Immunol* 131:2781–2783, 1983
105. FORD PM, KOSATKA I: A mechanism of enhancement of immune complex deposition following in situ immune complex formation in the mouse glomerulus. *Immunology* 43:433–438, 1981
106. STRIKER GE, MANNIK M, TUNG MY: Role of marrow-derived monocytes and mesangial cells in removal of immune complexes from renal glomeruli. *J Exp Med* 149:127–136, 1979
107. BARCELLI U, RABBMACHER R, OOI YM, OOI BS: Modification of glomerular immune complex deposition in mice by activation of the reticuloendothelial system. *J Clin Invest* 67:20–27, 1981
108. HAAKENSTAD AO, STRIKER GE, MANNIK M: The glomerular deposition of soluble immune complexes prepared with reduced and alkylated antibodies and with intact antibodies in mice. *Lab Invest* 35:293–301, 1976
109. CORNACOFF JB, HEBERT LA, SMEAD WL, VANAMAN ME, BIRMINGHAM DJ: The primate erythrocyte-immune complex clearing mechanism. *J Clin Invest* 71:236–247, 1983
110. HEBERT LA, ALLHISER CL, KOETHE SM: Some hemodynamic determinants of immune complex trapping by the kidney. *Kidney Int* 14:452–465, 1978
111. WARD DM, BLANTZ RC: Uptake of macromolecules by the glomerular mesangium is increased by angiotensin II *(abstract). Clin Res* 29:479, 1981
112. RAIJ L, KEANE WF, OSSWALD H, MICHAEL A: Mesangial function in ureteral obstruction in the rat. Blockade of the efferent limb. *J Clin Invest* 64:1204–1212, 1979
113. MANNIK M: Pathophysiology of circulating immune complexes. *Arth Rheum* 25:783–787, 1982
114. GAUTHIER VJ, MANNIK M, STRIKER GE: Effect of cationized antibodies in preformed immune complexes on deposition and persistence in renal glomeruli. *J Exp Med* 156:766–777, 1982
115. BARNES JL, RADNIK RA, GILCHRIST EP, VENKATACHALAM MA: Size and charge selective permeability defects induced in glomerular basement membrane by a polycation. *Kidney Int* 25:11–19, 1984
116. COUSER WG, SALANT DJ, MADAIO MP, ADLER S, GROGGEL GC: Factors influencing glomerular and tubulointerstitial patterns of injury in systemic lupus erythematosus. *Am J Kidney Dis* 2 (suppl 1):126–134, 1982
117. SALANT DJ, ADLER S, DARBY C, GROGGEL GC, RENNKE HG, DITTMER JE: Influence of antigen distribution on the mediation of immunologic glomerular injury. *Kidney Int* 27:938–950, 1985
118. COUSER WG, DARBY C, SALANT DJ, ADLER S, STEELE KM, STILMANT MM, LOWENSTEIN LM: Effect of antibody to glomerular basement membrane on protein excretion in the isolated perfused rat kidney. *Am J Physiol,* in press
119. SALANT DJ, CAPPARELL N, MADAIO MP, DARBY CM, STILMANT MM, COUSER WG: Altered glomerular permeability induced by $F(ab)_2$ and Fab' antibodies to rat renal tubular epithelial antigen. *Kidney Int* 21:36–43, 1981
120. YAAR M, FOIDART JM, BROWN KS, RENNARD SI, MARTIN GR, LIOTTA L: The Goodpasture-like syndrome in mice induced by intravenous injections of anti-type IV collagen and anti-laminin antibody. *Am J Pathol* 107:79–91, 1982
121. ROTHBARD S, WATSON RF: Comparison of reactions of antibodies to rat collagen and to rat kidney in the basement membranes of rat renal glomeruli. *J Exp Med* 129:1145–1161, 1969
122. SALANT DJ, BELOK S, MADAIO MP, COUSER WG: A new role for complement in experimental membranous nephropathy in rats. *J Clin Invest* 66:1339–1350, 1980
123. BAKER PJ, OCHI R, ADLER S, JOHNSON RJ, COUSER WG: C6 depletion abolishes proteinuria in experimental membranous nephropathy *(abstract). Clin Res* 33:475A, 1985
124. GROGGEL GC, SALANT DJ, DARBY C, RENNKE HG, COUSER WG: Role of the terminal complement pathway in the heterologous phase of anti-glomerular basement membrane nephritis in the rabbit. *Kidney Int* 27:643–651, 1985
125. ADLER S, BAKER PJ, PRITZL PM, COUSER WG: Presence of terminal complement components in complement-dependent experimental glomerulonephritis. *Kidney Int* 26:830–837, 1984
126. PERKINSON DT, BAKER PJ, COUSER WG, JOHNSON RJ, ADLER S: Membrane attack complex deposition in experimental glomerular injury. *Am J Pathol,* in press
127. BIESECKER G, KATZ S, KOFFLER D: Renal localization of the membrane attack complex in systemic lupus erythematosus nephritis. *J Exp Med* 151:1779–1794, 1981
128. FALK RJ, DALMASSO AP, KIM Y, TSAI CH, SCHEINMAN JI, GEWURZ H, MICHAEL AF: Neoantigen of the polymerized ninth component of complement. Characterization of a monoclonal antibody and immunohistochemical localization in renal disease. *J Clin Invest* 72:560–573, 1983
129. KOPP WC, BURRELL R: Evidence for antibody-dependent binding of the terminal complement component to alveolar basement membrane. *Clin Immunol Immunopathol* 23:10–21, 1982
130. SCHREIBER RD, MULLER-EBERHARD HJ: Complement and renal disease. *Contemp Issues Nephrol* 3:67–105, 1978
131. COCHRANE CG, UNANUE E, DIXON FJ: A role of polymorphoneuclear leukocytes and complement in nephrotoxic nephritis. *J Exp Med* 122:99–110, 1965
132. COCHRANE CG, MULLER-EBERHARD HJ, AIKEN BS: Depletion of plasma complement in vivo by a protein of cobra venom, its effect on various immunologic reactions. *J Immunol* 105:55–65, 1970
133. GANG NF, MAUTNER W, KALANT N: Nephrotoxic serum nephritis. II. Chemical, morphologic and functional correlates of glomerular basement membrane at the onset of proteinuria. *Lab Invest* 23:150–157, 1970
134. HENSON PM: Pathologic mechanisms in neutrophil-mediated injury. *Am J Pathol* 68:593–612, 1972
135. GOETZL E JR, AUSTIN KF: Active site chemotactic factors and the regulation of the human neutrophil chemotactic response, in *Antibiotics and Chemotherapy* (vol 19), edited by SORKIN E, Basel, Karger, 1974, pp 218–232
136. Ross GD: Analysis of the different types of leukocyte membrane complement receptors and their interaction with the complement system. *J Immunol Methods* 37:197–211, 1980
137. NAISH PF, THOMSON NM, SIMPSON IJ, PETERS DK: The role of polymorphonuclear leukocytes in the autologous phase of nephrotoxic nephritis. *Clin Exp Immunol* 22:102–109, 1975
138. SINDREY M, NAISH P: The mediation of the localization of polymorphonuclear leukocytes in glomeruli during the autologous phase of nephrotoxic nephritis. *Clin Exp Immunol* 35:350–355, 1979
139. HAWKINS D, COCHRANE CG: Glomerular basement membrane damage in immunological glomerulonephritis. *Immunology* 14:556–681, 1968
140. DAVIES M, BARRETT AJ, TRAVIS J, SANDERS E, COLES GA: The degradation of human glomerular basement membrane with purified lysosomal proteinases. Evidence for the pathogenic role of the

polymorphonuclear leukocyte in glomeruonephritis. *Clin Sci Mol Med* 54:233–240, 1978
141. COCHRANE CG, AIKIN BS: Polymorphonuclear leukocytes in immunologic reactions. The destruction of vascular basement membrane in vivo and in vitro. *J Exp Med* 124:733–752, 1966
142. FANTONE JC, WARD PA: Role of oxygen-derived free radicals and metabolites in leukocyte-dependent inflammatory reactions. *Am J Pathol* 107:397–418, 1982
143. FREEMAN BA, CRAPO JD: Biology of disease. Free radicals and tissue injury. *Lab Invest* 47:412–426, 1982
144. REHAN A, WIGGINS RC, JOHNSON KJ: Hydrogen peroxide induced proteinuria in rats caused by injection of phorbol myristate acetate (PMA) into the renal artery *(abstract). Kidney Int* 25:217, 1984
145. REHAN A, JOHNSON KJ, WIGGINS RC, KUNKEL RG, WARD PA: Evidence for the role of oxygen radicals in acute nephrotoxic nephritis. *Lab Invest* 51:396–403, 1984
146. SANO M: Participation of monocytes in glomerulonephritis in acute serum sickness of rabbit. *Acta Pathol Jpn* 26:423–433, 1976
147. THOMSON NM, HOLDSWORTH SR, GLASGOW EF, ATKINS RC: The macrophage in the development of experimental crescentic glomerulonephritis. *Am J Pathol* 94:223–236, 1979
148. JONES DB: Inflammation and repair of the glomerulus. *Am J Pathol* 27:991-1009, 1951
149. MONGA G, MAZZUCCO G, BARBIANO DIBELGIOJOSO G, BUSNACH G: The presence and possible role of monocyte infiltration in human chronic proliferative glomerulonephritis. *Am J Pathol* 94:271–281, 1979
150. BARNES JL, VENKATACHALAM MA: Role of platelets and polycationic mediators in glomerular vascular injury. *Semin Nephrol,* in press
151. SCHREINER GF, COTRAN RS, PARDO V, UNANUE ER: A mononuclear cell component in experimental immunological glomerulonephritis. *J Exp Med* 147:369–384, 1978
152. HOLDSWORTH SR, NEALE TJ, WILSON C: Abrogation of macrophage-dependent injury in experimental glomerulonephritis in the rabbit. Use of anti-macrophage serum. *J Clin Invest* 68:686–696, 1981
153. HOLDSWORTH SR, NEALE TJ: Macrophage-induced glomerular injury. Cell transfer studies in passive autologous antiglomerular basement membrane antibody-initiated experimental glomerulonephritis. *Lab Invest* 51:172–180, 1984
154. HOLDSWORTH SR: Fc dependence of macrophage accumulation and subsequent injury in experimental glomerulonephritis. *J Immunol* 130:735–739, 1983
155. TIPPING PG, NEALE TJ, HOLDSWORTH SR: T cell-macrophage interaction in antibody induced experimental glomerulonephritis *(abstract). Abst 9th Int Cong Nephrol,* 1984, p 264A
156. KREISBERG JI, WAYNE DB, KARNOVSKY MJ: Rapid focal loss of negative charge associated with mononuclear cell infiltration early in nephrotoxic serum nephritis. *Kidney Int* 16:290–300, 1979
157. NEILSON EG, PHILLIPS SM: Cell-mediated immunity in interstitial nephritis. IV. Anti-tubular basement membrane antibodies can function in antibody-dependent cellular cytotoxicity reactions: Observations on a nephritogenic effector mechanism acting as an informational bridge between the humoral and cellular immune response. *J Immunol* 126:1990–1993, 1981
158. SCHREINER GF, COTRAN RS, UNANUE ER: Macrophages and cellular immunity in experimental glomerulonephritis. *Springer Semin Immunopathol* 5:251–267, 1982
159. SCHARSCHMIDT LA, DUNN MJ: Prostaglandin synthesis by rat glomerular mesangial cells in culture. Effects of angiotensin II and arginine vasopressin. *J Clin Invest* 71:1756–1764, 1983
160. LOVETT DH, RYAN JL, KASHGARIAN M, STERZEL RB: Lysosomal enzymes in glomerular cells of the rat. *Am J Pathol* 107:161–166, 1982
161. LOVETT DH, STERZEL RB, RYAN JL, KASHGARIAN M: Neutral protease activity produced in vitro by cells of the glomerular mesangium. *Kidney Int* 23:342–349, 1983
162. BAUD L, HAGEGE J, SRAER J, RONDEAU E, PEREZ J, ARDAILLOU R: Reactive oxygen production by cultured rat glomerular mesangial cells during phagocytosis is associated with stimulation of lipoxygenase activity. *J Exp Med* 158:1836–1852, 1983
163. LOVETT DH, RYAN JL, STERZEL RB: A thymocyte-activating factor derived from glomerular mesangial cells. *J Immunol* 130:1796–1801, 1983
164. SEDOR JR, ABBOUD HE: Platelet activating factor stimulates oxygen radical release by cultured rat mesangial cells *(abstract). Kidney Int* 27:222, 1985
165. ADLER S, BAKER PJ, JOHNSON RJ, OCHI R, COUSER WG: Membrane attack complex (C5b-9) stimulates production of reactive oxygen species by rat mesangial cells *(abstract). Clin Res* 33:474A, 1985
166. SCHREINER GF, KIELY J-M, COTRAN RS, UNANUE ER: Characterization of resident glomerular cells in the rat expressing Ia-determinants and manifesting genetically restricted interactions with lymphocytes. *J Clin Invest* 68:920–931, 1981
167. ROCKLIN RE, LEWIS EJ, DAVID JR: In vitro evidence for cellular hypersensitivity to glomerular basement membrane antigens in human glomerulonephritis. *N Engl J Med* 283:497–501, 1970
168. FILLIT HM, READE SE, SHERMAN RL, ZABRISKI JB, VAN DERIJN I: Cellular reactivity to altered glomerular basement membrane in glomerulonephritis. *N Engl J Med* 298:861–868, 1978
169. DIXON FJ: What are sensitized cells doing in glomerulonephritis? *(editorial). N Engl J Med* 283:536–537, 1970
170. BAHN AK, COLLINS A, SCHNEEBERGER E, MCCLUSKEY R: A cell-mediated reaction against glomerular-bound immune complexes. *J Exp Med* 150:1410–1417, 1979
171. UNANUE ER, SCHREINER GF, COTRAN RS: A role of mononuclear phagocytes in immunologically induced glomerulonephritis, in *Immune Mechanisms in Renal Disease,* edited by CUMMINGS NB, MICHAEL AF, WILSON CB, New York, Plenum, 1983, pp 443–452
172. BOLTON WK, TUCKER FL, STURGILL BC: New avian model of experimental glomerulonephritis consistent with mediation by cellular immunity. *J Clin Invest* 73:1263–1276, 1984
173. CHANDRA M, TYSON T, STURGILL B, BOLTON K: Transfer of experimental autoimmune glomerulonephritis in chickens by sensitized cells *(abstract). Kidney Int* 27:208, 1985
174. COUSER WG, ADLER SA, BAKER PJ, JOHNSON RJ, PERKINSON DA: Mechanisms of immune complex formation and deposition in glomeruli. *Nephrology. Proc 9th Int Cong Nephrol,* edited by ROBINSON RR, Springer-Verlag, New York, 1984, pp 508–526
175. ABRASS CK, NIES KM, LOUIS JS, BORDER WA, GLASSOCK RJ: Correlation and predictive accuracy of circulating immune complexes with disease activity in patients with systemic lupus erythematosus. *Arthr Rheum* 23:273–282, 1980
176. CAIRNS SA, LONDON RA, MALLICK NP: Circulating immune complexes in idiopathic glomerular disease. *Kidney Int* 21:507–512, 1982
177. SOLLING J, OLSEN S: Circulating immune complexes in glomerulonephritis. *Clin Nephrol* 16:63–74, 1981
178. BRENNER BM: Nephrology Forum: Hemodynamically mediated glomerular injury and the progressive nature of kidney disease. *Kidney Int* 23:647–655, 1983
179. BLANTZ RC, WILSON CB: Acute effects of anti-glomerular basement membrane antibody on the process of glomerular filtration in the rat. *J Clin Invest* 58:899–911, 1976
180. BLANTZ RC: Physiologic approaches to the mechanisms of glomerular immune injury (chapter 5), in *Immune Mechanisms in Renal Disease,* edited by CUMMINGS NB, MICHAEL AF, WILSON CB, Plenum, New York, 1983, pp 83–94
181. BALDWIN DS, GLUCK MC, SHACT RG, GALLO G: The long-term course of poststreptococcal glomerulonephritis. *Ann Intern Med* 80:342–358, 1974
182. KAPLAN EL, ANTHONY BF, CHAPMAN SS, WANNAMAKER LW: Epidemic acute glumerulonephritis associated with Type 49 streptococcal pyoderma. I. Clinical and laboratory findings. *Am J Med* 48:9–27, 1970
183. REES AJ: The HLA complex and susceptibility to glomerulonephritis. *Plasma Ther Transfus Technol* 5:455–470, 1984
184. REES AJ, PETERS DK, AMOS N, WELSH KJ, BATCHELOR JR: The influence of HLA-linked genes on the severity of anti-GBM antibody-mediated nephritis. *Kidney Int* 26:444–450, 1984
185. BERTHOUX FC, LAURENT B, LE PETIT J-C, GENIN C, BROUTIN F, TOURAINE F, HASSAN AA, CHAMPAILLER A: Immunogenetics and immunopathology of human primary membranous glomerulo-

nephritis: HLA-A, B, DR antigens; functional activity of splenic macrophage Fc-receptors and peripheral blood T-lymphocyte subpopulations. *Clin Nephrol* 22:15–20, 1984
186. SASAGUKI T, HAYASE R, IWANOTO I, TSUCHIDA H: HLA and acute poststreptococcal glomerulonephritis. *N Engl J Med* 301:1184–1185, 1979
187. LAYRISSE Z, RODRIGUEZ-ITURBE B, GARCIA-RAMIREZ R, RODRIGUEZ A, TIWARI J: Family studies of the HLA system in acute post-streptococcal nephritis. *Hum Immunol* 7:177–185, 1983
188. JORDAN SC, LEMIRE JM: Acute glomerulonephritis. Diagnosis and treatment. *Pediatr Clin North Am* 29:857–873, 1982
189. CAMERON JS, VICK RM, OGG CS, SEYMOUR WM, CHANTLER C, TURNER DR: Plasma C3 and C4 concentrations in the management of glomerulonephritis. *Br Med J* 3:668–672, 1973
190. LEWIS EJ: Patterns of circulating complement in renal diseases. *Annu Rev Med* 30:445–455, 1979
191. TINA LV, D'ALBORA JB, ANTONOVYCH TT, BELLANTI JA, CALCHENO PL: Acute glomerulonephritis associated with normal serum β1C-globulin. *Am J Dis Child* 115:29–36, 1968
192. MCLEAN RH, SCHRAGER MA, ROTHFIELD NF, BERMAN MM: Normal complement in early poststreptococcal glomerulonephritis. *Br Med J* 1:1326–1328, 1977
193. NEWGARTEN J, GALLO GR, BALDWIN DS: Glomerulonephritis in bacterial endocarditis. *Am J Kidney Dis* 3:371–379, 1984
194. BEAUFILS M: Nephrology Forum: Glomerular disease complicating abdominal sepsis. *Kidney Int* 19:609–618, 1981
195. WILSON CB: Immunopathologic evaluation of renal disease, in *Renal Disease* (4th ed), edited by BLACK AK, JONES NF, Oxford, Blackwell, 1979, p 158
196. GREKAS D, MORLEY AR, WILKINSON R, KERR DNS: Isolated C3 deposition in patients without systemic disease. *Clin Nephrol* 21:270–274, 1984
197. BAKER PJ, ADLER S, YANG Y, COUSER WG: Complement activation by heat-killed human kidney cells: formation, activity and stabilization of cell bound C3 convertases. *J Immunol* 133:877–881, 1984
198. HANSCH GM, SEITZ M, MARTINOTTI G, BETZ M, RAUTEBERG EW, GEMSA D: Macrophages release arachidonic acid, prostaglandin E_2 and thromboxane in response to late complement components. *J Immunol* 133:2145–2150, 1984
199. LOVETT D, HANSCH G, RESCH K, GEMSA D: Activation of glomerular mesangial cells (MC) by terminal complement components: *Immunobiology* 168:34–35, 1984
200. POLLAK VE, DOSEKUN AK: Evaluation of treatment in lupus nephritis: effects of prednisone. *Am J Kidney Dis* 2(Suppl 1): 170–177, 1982
201. COUSER WG: Rapidly progressive glomerulonephritis (edit rev). *Am J Nephrol* 2:57–69, 1982
202. Collaborative Study of the Adult Idiopathic Nephrotic Syndrome: A controlled study of short-term prednisone treatment in adults with membranous nephropathy. *N Engl J Med* 301:1301–1309, 1979